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Evaluation of LDH and G6-PDH Activities in Auditory Relay Centers of Streptozotocin-induced Diabetic Wistar Rats

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ABSTRACT

This study was designed to assess the direct effects of chronic hyperglycemia on the hearing pathway with reference to the auditory relay nuclei— the medial geniculate body (MGB) and inferior colliculus (IC)— based on the biochemical assay of carbohydrate metabolic pathways using marker enzymes as reference points. A total of 39 healthy adult Wistar rats (of both sexes) weighing200- 220g were used in this study. Twenty-four of them were rendered diabetic by a single STZ intraperitoneal injection of 70 mg/kg body weight; fifteen were used as control. Activities of lactate dehydrogenase (LDH) for the glycolytic pathway and glucose-6-phosphate dehydrogenase (G6-PDH) for the pentose phosphate pathway were assessed at post-induction periods P4, P7 and P10 (in weeks). The activities of the enzymes decreased ($P<0.05$) through the periods of assessment as revealed by the biochemical assay results (tables 2 & 3). The study offers fresh evidence that STZ-induced diabetes mellitus can cause a derangement in the pathways of carbohydrate metabolism in the medial geniculate body and inferior colliculus and may be implicated as one of the factors responsible for the mechanism of hearing impairment experienced in diabetic conditions.

Key words: streptozotocin (STZ), diabetes mellitus (DM), medial geniculate body (MGB), inferior colliculus (IC), lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), glucose-6-phosphate dehydrogenase (G6-PDH)

INTRODUCTION

The incidence of diabetic neuropathy has been increasing.[1-12] Studies have shown that oxidative stress, probably due to increased oxidant production and /or decreased antioxidant activity forms a critical underlying mechanism.[12,13] Reduced nicotinamide adenine dinucleotide phosphate (NADPH) has been found to be the principal intracellular reductant and its production is mainly dependent on glucose-6-phosphate dehydrogenase (G6-PDH) activity. Saha[14] and Zang[15] reported that G6-PDH activity is inhibited by high blood glucose levels (hyperglycemia). This is achieved through the activation of protein kinase A (PKA) and subsequent phosphorylation of the G6-PDH. Inhibition of G6-PDH activity further decreases the production of NADPH. A concomitant increase in lipid peroxidation is a major feature in the hyperglycemic condition, with all the changes being ameliorated by insulin treatment or other measures to correct hyperglycemia.[15]

In addition to the well-known and described complications of diabetes, viz, retinopathy,[16] and both autonomic and peripheral neuropathy,[13, 17-20] various changes characterized by numerous, morphological and molecular abnormalities have been reported[9,21,22] as complications of diabetes mellitus in the auditory pathway.

This study has investigated some of the effects of streptozotocin-induced diabetes mellitus on the pathways of glucose metabolism in the diencephalic and mesencephalic auditory relay centers (MGB & IC) based on the activities of marker enzymes such as lactate dehydrogenase and glucose-6-phosphate dehydrogenase.

MATERIALS AND METHODS

Thirty-nine adult Wistar rats weighing averagely 220g ± were cared for in compliance with the principles of laboratory animal care. They were provided with standard rat pellets (Ladokun Feeds, Oyo

State, Nigeria) and water ad libitum. All rats were carefully assessed, screened and confirmed to be free of any pathological condition before starting the experiment and maintained under a constant 12-hour light and dark cycle. They were randomly assigned into four groups as control (n=15), treated I (4 weeks), treated II (7 weeks) and treated III (10 weeks). Streptozotocin, (Sigma Inc. USA), was procured and used in the production of diabetic models. Rats in the treatment groups (n=24) each received a single dose of 70 mg/kg body weight of streptozotocin dissolved in citrate buffer (pH 4.5). One(1) ml of dissolved STZ was injected intraperitoneally into each rat. The control rats (n=15) received an equivalent volume of citrate buffer only. Glycemic levels were measured with the glucose oxidase – peroxidase[23] reagent [GOD-POD – RANDOX KIT, CAT NO. /KAT NR. - G12623, GL2614, GL 2610]. The rats were weighed twice a week.

At intervals of 4 weeks (T1), 7 weeks (T2), 10 weeks (T3), hyperglycemic rats (glucose level >125±16.06mg/dl – table 1) were sacrificed by cervical dislocation. The brains were quickly removed and rapidly weighed after which the diencephalic and mesencephalic auditory relay centers were rapidly homogenized for bioenzymatic assays. Cryostat sections were cut at 20μm for histochemical staining and examination under a light microscope. All the data are expressed as mean ± SD of the number of experiments (n=24). Statistical difference was evaluated using the one-way analysis of variance (ANOVA) on the SPSS version 10.0.*Differences between means were considered statistically significant at $P<0.05$.

RESULTS

Treatment with a single dose of 70 mg/kg body weight of STZ caused a significant decrease in the body weight of the treated animals (220 ± -9.01 at zero hour vs. 166.6±9.72 at week 10, $P<0.05$) (table 1). The blood glucose levels were significantly increased in treated rats (56.5 ± 11.5mg/dl at zero hour when compared with 304.5±7.14mg/dl at week 10, $P<0.05$). In the control rats, blood glucose levels fluctuated between 64.1±5.22mg/dl at zero hour and 67.9±3.54mg/dl at week 10 (table 1).

DISCUSSION

Results obtained in the present study show that STZ-induced hyperglycemia leads to significant decreases in the activities of the marker enzymes of the carbohydrate metabolic pathways. While the body weight of the treated rats reduced significantly, the blood glucose levels showed a 3-fold increase (table 1).

Table 1. General characteristic of the rats (average body weight (g) and blood glucose levels (mg/dl))

	Average body weight (g)		Average blood glucose (mg/dl)	
Time	Control(n=15)	Treated(n=24)	Control(n=15)	Treated(n=24)
0 hour	220 ± 18.15	220 ± 9.01	64.1 ± 5.22	56.6 ± 11.58
3 hours	220 ± 14.15	194 ± 7.01	65.4 ± 5.21	125.3 ± 16.06
24 hours	213 ± 17.18	193 ± 7.96	66.3 ± 5.10	99.9 ± 6.83
2 weeks	225 ± 18.71	193 ± 7.92	66.0 ± 4.44	135.8 ± 15.85
3 weeks	228 ± 20.49	192 ± 7.86	65.2 ± 5.33	158.9 ± 17.87
4 weeks	233 ± 22.36	190 ± 7.95	67.2 ± 5.24	184.1 ± 15.04
5 weeks	235 ± 22.63	183 ± 5.90	67.4 ± 4.60	220.3 ± 18.85
6 weeks	235 ± 9.71	173 ± 8.84	68.1 ± 3.35	243.6 ± 17.91
7 weeks	237 ± 11.67	170 ± 10.13	67.8 ± 4.24	260.2 ± 13.87
8 weeks	239 ± 18.76	168 ± 9.58	68.6 ± 3.75	271.9 ± 10.83
9 weeks	240 ± 6.58	167 ± 9.46	68.6 ± 3.24	284.4 ± 10.03
10 weeks	242 ± 14.40	166.6 ± 9.72	67.9 ± 3.54	304.5 ± 7.14

Values are expressed as mean ± SD of 24 rats in treated groups and 15 rats in the control.

Table 2. Effect of STZ-induced diabetes on the relative wet brain weight of rats (g)

	Average relative wet brain weight of rats	
Time	Control (n=15)	Treated (=24)
Week 4	1.758 ± 0.02	1.574 ± 0.04
Week 7	1.743 ± 0.02	1.480 ± 0.03
Week 10	1.735 ± 0.008	1.414 ± 0.02

Values are expressed as mean ± SD of 24 rats in treated groups and 15 rats in control group ($P<0.05$).

Table 3. Effect of STZ-induced diabetes on lactate dehydrogenase activity (μmol/min/mg)

Time	Medial geniculate body		Inferior colliculus	
	Control(n=15)	Treated(n=24)	Control(n=15)	*Treated(n=24)*
Week 4	1.243 ± 0.02	0.397 ± 0.11	1.229 ± 0.18	0.512 ± 0.04
Week 7	1.233 ± 0.02	0.352 ± 0.10	1.84 ± 0.14	0.478 ± 0.00
Week 10	1.229 ± 0.01	0.335 ± 0.11	1.71 ± 0.14	0.453 ± 0.02

Data are expressed as mean ± SD of 24 rats in treated groups and 15 rats i control.

Table 4. Effect of STZ-induced diabetes on glucose-6-phosphate dehydrogenase activity (μmol/min/mg)

Time	Medial geniculate body		Inferior colliculus	
	Control(n=15)	Treated(n=24)	Control(n=15)	Treated(=24)
Week 4	2.700 ± 0.05	0.593 ± 0,05	1.484 ± 0.04	0.956 ± 0.03
Week 7	2-610 ± 0.05	0.487 ± 0.01	1.479 ± 0.02	0.911 ± 0.06
Week 10	2.501 ± 0.01	0.443 ± 0.03	1.475 ± 0.03	0.858 ± 0.08

Data are expressed as mean ± SD of 24 rats in treated groups and 15 rats control group ($P< 0.05$).

* SPSS, Cary, NC, USA.

The activities of both G6-PDH and LDH appeared to have been more affected in the medial geniculate body than what was obtained in the inferior colliculus (tables 3 & 4) suggesting a tissue specific dependence effect and inherent ability to adapt to the effects of hyperglycemic assaults.

The incidence of diabetic neuropathy has been increasing. Studies have shown that oxidative stress, probably due to increased oxidant production and /or decreased antioxidant activity forms a critical underlying mechanism. Reduced nicotinamide adenine dinucleotide phosphate (NADPH) has been found to be the principal intracellular reductant and its production is mainly dependent on glucose-6-phosphate dehydrogenase (G6-PDH) activity.[24,15] Zhang et al.[15] reported that G6-PDH activity is inhibited by high blood glucose levels (hyperglycemia). This is achieved through the activation of protein kinase A (PKA) and subsequent phosphorylation of the G6-PDH. Inhibition of G6-PDH activity further decreases the production of NADPH. A concomitant increase in lipid peroxidation forms a major feature in those conditions, with all the changes being ameliorated by insulin treatment or other measures to correct hyperglycemia. The oxidative stress induced by chronic hyperglycemia which causes inhibition of G6-PDH activity further accounts for the morphological changes observed in the auditory relay centers, viz the medial geniculate body and the inferior colliculus. G6-PDH is critical in the regeneration of NADPH, a coenzyme that is essential for protection against and repair of oxidative damage and also plays an important role in maintaining the proper 3-dimensional structure of proteins in the cell membranes.[25] As the first and rate-limiting enzyme in the pentose phosphate pathway the role of G6-PDH becomes critical to the integrity of the cells (in this study, the neurons) as the entire antioxidant system and other processes requiring reduction rely on the adequate supply of NADPH. A decrease in G6-PDH activity and a subsequent decrease in NADPH makes the cells very sensitive to oxidant damage due to oxidative stress. This leads to and explains the high percentages of cell death observed in the experimental rats compared to the control animals; DM induced rats actually became worse with prolonged untreated and uncontrolled condition of induced diabetes.

Information on the particular enzyme activity in relation to STZ-induced diabetes in the MGB and IC is sparse. Whereas most authors have focused their research work on the measurement of the brainstem auditory evoked responses (BAER)[5, 2, 6, 10, 11,26, 27] this study has attempted to assess, at the molecular level, the mechanism of action whereby diabetes mellitus is able to induce the hearing impairment associated with it.

As against the report of Lisowska et al,[28] who recorded that hearing impairment in diabetic patients is usually mild and sub clinical (in human subjects), the results of this study show that there are more severe assaults to the relay centers of the auditory pathway at the molecular level. This effect is further reflected in histological results where treated sections presented a widespread breakdown of the cellular integrity of the relay centers (unpublished). Morphometric studies have also shown an appreciable reduction in the neuronal enumeration of the relay centers, while the population of the glial cells was on the increase (unpublished).

Association between diabetes and the auditory system has been debated since it was first reported by Jordao in 1857.[29] Different types of hearing impairment have been reported in diabetic patients such as progressive, gradual, bilateral sensorineural loss, especially affecting the elderly and high frequencies of sounds.

This may be similar to presbycusis, but with more severe impact than that due to advancing age.[1,4,29,30] There are reports of early sensorineural loss[1] and hearing loss in low and medium frequencies.[31-32] Dalton[33] also reported unilateral sudden hearing loss secondary to diabetes mellitus affection.

Experimental studies using STZ and alloxan-induced diabetic rat models revealed the occurrence of microangopathy in the inner ear of the rats,[34] loss of outer hair cells, especially in rats that were simultaneously exposed to noise.[35] However, these observations were disputed by Nageris et al,[29] who argued that those models did not reflect the real physiological mechanism of diabetes in human beings who would probably show genetic affection.

The underlying causes of diabetic complications have been attributed to hyperglycemia which results in oxidative stress, alterations in enzyme activities, protein glycosylation and several structural changes. The onset and progression of these changes can, however, be prevented by strict glycemic control.[36] Increased accumulation of advanced glycation end products, increased flux through the polyol pathway, formation of oxidants, alterations in enzyme activities, amongst other complications, have received sufficient experimental evidence to support their role in diabetic complications.[12, 37-39]

Alterations in the enzymes of carbohydrate metabolism pathways (responsible for supplying energy to the cells) have featured prominently in diabetes. This study indicated that the nature of alterations depends on the specific tissue. Oxidative stress impacts directly on the activities of the enzymes, causing a significant ($P < 0.05$) reduction and subsequently reduced supply of energy to the cells which leads to eventual death of the cells.

CONCLUSION

So far, the molecular involvement of the auditory relay centers in diabetic complications has not been clarified. This study was undertaken to investigate their role in auditory disorders arising from hyperglycemia occasioned by STZ-induced diabetes. The results of this study offers fresh evidence that STZ-induced diabetes mellitus can cause a derangement in the pathways of carbohydrate metabolism in the medial geniculate body and inferior colliculus and may be implicated as one of the factors responsible for the mechanism of hearing impairment experienced in diabetic conditions.

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